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PRODUCT DATASHEET

ChemiScreen™ CCR1 Chemokine Membrane Preparation

CATALOG NUMBER:	HTS005M	**QUANTITY:**	200 units
LOT NUMBER:		**VOLUME/CONCENTRATION:**	1 mL, 1 mg/mL

BACKGROUND:

CCR1 is a GPCR that binds to a variety of CC ligands, including MIP-1α, RANTES, MCP-3, HCC-1, HCC-2, HCC-4, and MPIF-1 (Olson and Ley, 2002). Lymphocytes, macrophages, dendritic cells, and GM-CSF-activated neutrophils express CCR1 (Kaufmann *et al.*, 2001; Cheng *et al.*, 2001). Two selective, non-peptide small molecule antagonists of CCR1, BX-471 and CP-481,715 have been synthesized (Gladue *et al.*, 2003; Liang *et al.,* 2000). Pharmacological and genetic targeting of CCR1, either alone or in combination with cyclosporin A, reduces cardiac and renal allograft rejection (Gao *et al.,* 2000; Horuk *et al.,* 2001a; Horuk *et al.,* 2001b), allergic encephalomyelitis (Liang *et al.,* 2000), and renal fibrosis (Anders *et al.,* 2002) in experimental models. CCR1 Membrane Preparations are ideal for screening molecules that disrupt interactions between CCR1 and its ligands. The CCR1 Membrane Preps bind to MIP-1α with a Kd of 1.8 nM, and yield greater than a 10-fold signal-to-background ratio with [^{125}I]-MIP-1α at a concentration of 0.1 nM.

APPLICATIONS:

Radioligand Binding Assay

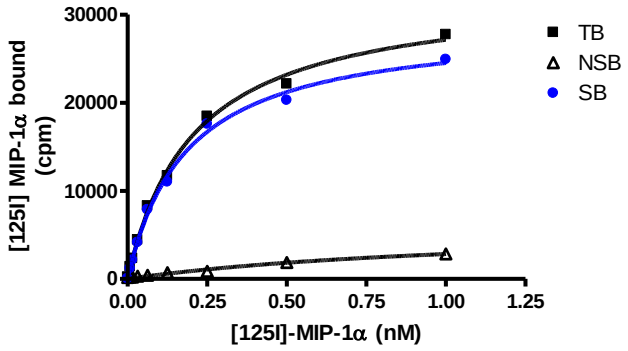

Figure 1. Saturation Binding for CCR1. 5 μg/well of CCR1 Membrane Preparation were incubated with increasing amounts of [^{125}I]-MIP-1α in the absence (total binding, TB) or presence (nonspecific binding, NSB) of an excess of unlabeled MIP-1α. Specific binding (SB) was determined by subtracting NSB from TB. The sample data are from a representative lot.

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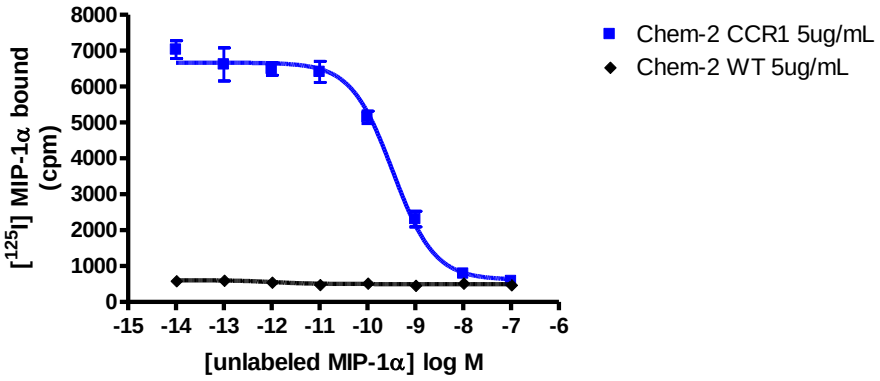

Figure 2. Competition binding of MIP-1α to CCR1 Membrane Preparations. CCR1 Membrane Preparation (5 µg/well) or Wild-Type Chem-2 Membrane Preparation (catalog # HTS000MC2) at10 µg/well were incubated with 0.1 nM [^{125}I]-MIP-1α and increasing concentrations of unlabeled MIP-1α. Greater than 10-fold signal to background was obtained with the CCR1 Membrane Preparation, whereas negligible signal was obtained with the Wild-Type Chem-2 Membrane Preparation. Representative sample data.

SPECIFICATIONS: 1 unit = 5 µg
B_{max} for [^{125}I]MIP-1α binding: 3.3 pmol/mg protein
K_d for [^{125}I] MIP-1α binding: ~ 0.02 nM
Signal:background: >10-fold

TRANSFECTION: Full-length human CCR1 cDNA (Accession Number: L09230)

HOST CELLS: Chem-2.

RECOMMENDED ASSAY CONDITIONS: Membranes are mixed with radioactive ligand and unlabeled competitor (see Figures 1 and 2 for concentrations tested) in binding buffer in a nonbinding 96-well plate, and incubated for 1-2 h. Prior to filtration, a GF/C 96-well filter plate is coated with 0.33% polyethyleneimine for 30 min, then washed with 50mM HEPES, pH 7.4, 0.5% BSA. Binding reaction is transferred to the filter plate, and washed 3 times (1 mL per well per wash) with Wash Buffer. The plate is dried and counted.

Binding buffer: 50 mM Hepes, pH 7.4, 5 mM $MgCl_2$, 1 mM $CaCl_2$, 0.2% BSA, filtered and stored at 4°C

Radioligand: [^{125}I] MIP-1α (Perkin Elmer # NEX298)

Wash Buffer: 50 mM Hepes, pH 7.4, 500mM NaCl , 0.1% BSA, filtered and stored at 4°C.

One package contains enough membranes for at least 200 assays (units), where a unit is the amount of membrane that will yield greater than 10-fold signal:background with ^{125}I-labeled MIP-1α at 0.1 nM.

PRESENTATION: Liquid in packaging buffer: 50 mM Tris pH 7.4, 10% glycerol and 1% BSA with no preservatives.
Packaging method: Membrane proteins were adjusted to the indicated concentration in 1 ml packaging buffer, rapidly frozen, and stored at -80°C.

STORAGE/HANDLING: Store at –70°C. Product is stable for at least 6 months from the date of receipt when stored as directed. Do not freeze and thaw.

REFERENCES:

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